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Full Length Article

Status of Insecticide Resistance in Field-collected Populations of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae)

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Abstract

The first monitoring of the resistance to two organophosphates (methidathion and ethion) and one pyrethroid (cypermethrin) insecticides presented by three field collections of different *Rhynchophorus ferrugineus* (Olivier) populations was performed in this study. The LD_{50} doses of three insecticides against newly moulted eighth-instar *R. ferrugineus* larvae were estimated through toxicity bioassays, in which the larvae were fed on artificial diet supplemented with different individual doses of cypermethrin, methidathion or ethion. A population of *R. ferrugineus* collected from Wadi Ad-Dawasir was chosen as the reference strain, because of its susceptibility to each of the tested insecticide. Compared with the susceptible strain, populations sampled from Al-Ahsa and Al-Qatif showed 8.72 and 4.51-folds increase in resistance to cypermethrin and 3.77 and 2.85-fold increases in resistance to ethion, respectively. The LD_{50} values of cypermethrin (1.62 ppm), methidathion (3.15 ppm) and ethion (9.12 ppm) in the susceptible population (Wadi Ad-Dawasir) were used to investigate the feeding performances and physiological impacts on eighth-instar *R. ferrugineus* larvae. The larvae showed no resistance to the most potent insecticide (methidathion) resulting highest growth reduction. However, moderate resistance was observed from red palm weevil population collected from Al-Qatif and Al-Ahsa against cypermethrin resulting lowest reduction in the efficacy of conversion of ingested food (ECI) and digested food (ECD). In addition, glutathione-S-transferase (GST) activity assay further strengthen our findings. Results suggest that enhanced GST activity likely contributes to cypermethrin and ethion resistance exhibited by the *R. ferrugineus* populations sampled from Al-Qatif and Al-Ahsa. Toxicity bioassays, dietary utilization experiments and GST assays examined in this study support the use of methidathion against *R. ferrugineus* as the most potent insecticide against all the sampled populations. However, cypermethrin and ethion resistance appears to be evolving in the *R. ferrugineus* populations from Al-Qatif and Al-Ahsa, justifying the concern of farmers regarding the low efficacy of insecticides.

Keywords: Growth indices; Glutathione S-transferase; Insecticide susceptibility; Resistance monitoring; Red palm weevil

Introduction

Rhynchophorus ferrugineus (Olivier) (Coleoptera: Curculionidae), also known as the red palm weevil (RPW), is an insect endemic to India and considered the most serious tissue-boring pest of palms. Severe RPW infestations have been reported on *Cocos nucifera*, *Phoenix dactylifera* and *Phoenix canariensis* (Gadelhak and Enan, 2005; El-Mergawy *et al*., 2011; Hussain *et al*., 2013a). Recent statistics have shown that RPW infestations may cause severe economic losses that range from 1% to 5% (5.18 to 25.92 million USD), and the indirect losses increase these figures by several folds (El-Sabea *et al.,* 2009). The most destructive stage of *R. ferrugineus* is the legless, creamy white larval stage (grubs), which chews the tender, soft tissues of the palms and moves toward the interior of the plant. During severe infestations, this feeding pattern results in toppling of the palm crown, which ultimately leads to the collapse of the infested palm (Hussain *et al.*, 2013b). The management of RPW presents a tremendous challenge because of its cryptic life cycle, and the application of synthetic insecticides via soil treatments, tree fumigation, frond axil filling, trunk injections, wound dressing and crown drenching of the infested palms remains the main strategy for RPW control (Hussain *et al.,* 2013b).

In the past, the effectiveness of a number of

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insecticides from different groups against RPWs has been evaluated. A chloronicotinyl nitroguanidine insecticide, imidacloprid when compared with oxamyl was found to be highly potent against all of the studied stages of RPW (Cabello *et al.,* 1997). Laboratory and greenhouse assays conducted by Kaakeh (2006) also showed that imidacloprid presents good efficacy against different RPW stages. The findings reported by Abdul-salam *et al.* (2001) showed that the exposure of different stages of RPW to fipronil resulted in 100% larval and adult mortality. In Saudi Arabia, Abo-El-Saad *et al.* (2001) investigated the efficacy of pyrethroids against larvae and adults. Their results revealed that cypermethrin was more effective against larvae and adults, whereas permethrin, deltamethrin and fenvalerate were found to be ineffective. Study evaluating the efficacy of organophosphates insecticide (pirimiphos-methyl) showed high efficacy compared with chlorpyrifos against adult weevils, whereas oxydemeton-methyl showed greater insecticidal activity than chlorpyrifos against larvae (Ajlan *et al.,* 2000). Experiments using carbamates, such as carbaryl and a mixture of carbaryl and piperonyl butoxide (PBO), have suggested that *R. ferrugineus* larvae are more susceptible to carbamates than adults (Al-Rajhy *et al.,* 2005). The neonicotinoid thiamethoxam has also been approved for use as RPW-controlling insecticide in some countries, including the Republic of Cyprus. In Spain, laboratory experiments conducted by Llácer and Jacas, (2010) have recommended the use of aluminium phosphide as a quarantine measure against RPW. In Pakistan, a study was conducted to assess the efficacy of ten different insecticides. The results showed that spirotetramat, fipronil, chlorpyrifos and methidathion effectively control RPW infestations of date palms under field conditions (Shar *et al.,* 2012). We recently evaluated the efficacy of ten different insecticides against the mid-gut cell line of RPWs and found that emamectin benzoate was a highly toxic insecticide that resulted in 92% cell mortality and 74% growth inhibition (Al-Jabr *et al.,* 2013).

In the Gulf region, particularly in Saudi Arabia, the ineffectiveness of the currently available insecticides is recently complained by the farmers. The ineffectiveness might because of the development of insecticide resistance. However, the mechanisms underlying insecticide resistance in *R. ferrugineus* are poorly understood. The scarcity of knowledge and the limited explorations of the mechanisms of insecticide resistance in RPW populations were taken into account in the design of the current investigation. We selected three different frequently used insecticides (cypermethrin, ethion and methidathion) and evaluated their toxicity against RPW populations collected from three different areas of the Kingdom of Saudi Arabia. In addition, we provide the first investigation of the impact of the selected insecticides on the growth of different populations of *R. ferrugineus* and the GST activity associated with the observed insecticide resistance.

Materials and Methods

Maintenance of Red Palm Weevils

Adult RPWs were collected from three different localities of Saudi Arabia: the Al-Ahsa, Wadi Ad-Dawasir, and Al-Qatif regions. These three populations were separately maintained on pineapples in perforated cages (57.5 × 29 × 58 cm) at 30±1°C. Second-instar grubs were carefully shifted to an artificial diet (consisting of 45 g/L wheat flour, 45 g/L corn flour, 45 g/L yeast, 1.6 g/L sorbic acid, 4 g/L ascorbic acid, two capsules of pharmaton per litre, 500 mg/L tetracycline, and 17.5 g/L agar in 1000 mL of distilled H_2O) in perforated, transparent plastic cups with a capacity of 400 mL (Hussain *et al*., 2015). Larvae from each regional population were reared separately at 30 ± 1°C with 75% ± 5% relative humidity in an incubator (Sanyo). The larvae were provided with fresh food daily.

Insecticides and Chemical Reagents

Formulated cypermethrin (Cyprine 100 EC), ethion (Ethio 500 EC) and methidathion (Cobracide 400 EC) were purchased from Astra Chemicals (Saudi Arabia). A glutathione-S-transferase (GST) assay kit (CS0410-1KT) was purchased from Sigma-Aldrich (USA).

Toxicity Bioassays to Determine the Levels of Resistance

Preliminary laboratory toxicity bioassays (data not shown) were performed to identify a range of doses to be used for determining the LD_{50} values of each of the studied insecticides. Based on these preliminary laboratory toxicity bioassays, five doses of each insecticide were prepared using ddH_2O. Separate preparations of artificial diets were prepared using individual insecticide solutions. Among each of the three RPW populations studied, newly moulted eighth-instar larvae were allowed to feed on diets containing different doses of measured quantity of cypermethrin, methidathion or ethion. A control treatment diet was prepared using ddH_2O. All of the treatments were incubated at 30 ± 1°C with 75% ± 5% relative humidity in an incubator (Sanyo). Five replicates were prepared for each experimental unit, and each replicate consisted of 25 larvae. The whole study was repeated over time. The mortality data were recorded daily until 100% mortality. A larva was considered dead if no signs of movement were observed. Abbott's formula was used to correct the percent larval mortality of the RPWs (Abbott, 1925). The corrected angularly transformed percent mortality data were analysed by one-way analysis of variance (ANOVA). The means were compared by Fisher's Least-Significant Difference (LSD) test (SAS Institute, 2000). The median lethal doses (LD_{50}s) of methidathion, cypermethrin, and ethion against each population of RPWs were separately calculated through Probit analysis. Because there is no standard susceptible laboratory strain of *R. ferrugineus*, the population with the lowest LD_{50} values was treated as the susceptible strain. The Resistance Ratio 50 (RR_{50}) values for cypermethrin, ethion and methidathion were calculated by dividing the LD_{50} values of the suspected resistant population by the LD_{50} value of the susceptible population.

Impact of Insecticides on the Growth and Development of *R. ferrugineus* Larvae

Newly moulted eighth-instar RPW larvae were fed on artificial diets supplemented with either 1.62 ppm cypermethrin, 3.15 ppm methidathion or 9.12 ppm ethion. These doses were determined from the LD_{50} values of the susceptible RPW population (Wadi Ad-Dawasir). A control treatment was prepared using ddH_2O. All of the experimental units were incubated at 30 ± 1°C with 75%±5% relative humidity in an incubator (Sanyo). The initial weight of the artificial diet offered to the larvae and the food remaining after 72 h were measured using an analytical balance. Furthermore, the frass produced over 72 h and the initial and final weights of the larvae were also measured. Five replicates were prepared for each experimental unit, and each replicate consisted of 25 larvae. The whole study was repeated over time. The insect, diet and frass weights were used to determine the feeding performance by calculating the efficacy of conversion of ingested food [ECI = 100 × dry weight gained by the larva/dry weight of food consumed] and the efficacy of conversion of digested food [ECD = weight gained by the larva/(food ingested – dry weight of frass)] (Hussain *et al*., 2009; Hussain *et al*., 2015). The data on the growth indices were analysed by one-way ANOVA. The means were compared using Fisher's LSD test (SAS Institute, 2000).

Assay of the Activity of the Detoxification Enzyme Glutathione-S-transferase (GST)

The activities of GST were calculated as described by Habig and Jakoby, (1981) using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. Newly moulted eighth-instar RPW larvae were fed separate diets containing 1.62 ppm cypermethrin, 3.15 ppm methidathion or 9.12 ppm ethion. After 72 h of feeding, the larvae were dissected in saline, and their mid-guts were separated. Homogenates containing 10% (w/v) mid-guts were prepared in cold 50 mM potassium phosphate buffer (pH 7.0) using a Teflon pestle-glass homogenizer. The homogenates were centrifuged (12,000 *g*) for 10 min at a low temperature (5°C). Five replicates were prepared. The supernatants served directly as the enzyme source. Four microliters of the enzyme source were incubated with 196 µL of substrate at 25°C according to the instructions provided by the manufacturer (Sigma Aldrich). After gentle shaking within a microplate reader, the changes in absorbance were recorded at 340 nm. Because we used a 96-well plate, we determined the extinction coefficient to be 5.3 mM^{-1} cm^{-1}. The absorbance was recorded after every minute for a period of 6 min. The specific GST activity data for each test group relative to the control group were analysed by one-way ANOVA. The means were compared using Fisher's LSD test (SAS Institute, 2000).

Results

Resistance Detection and Susceptibility

The evaluation of RPW populations sampled from different areas of the Kingdom of Saudi Arabia was variable, showing high degrees of contrast among the populations. The highest LD_{50} value (34.34 ppm) was found for the Al-Ahsa population, and this value is 3.77-fold higher that of the LD_{50} value obtained for the most susceptible population from Wadi Ad-Dawasir. The susceptible population had the lowest LD_{50} value (9.12 ppm) (Table 1). The analysis of cypermethrin showed variable levels of sensitivity. The population from Al-Ahsa was found to be resistant to cypermethrin, exhibiting an LD_{50} value of 14.13 ppm, which is 8.72-fold higher than that of the Wadi Ad-Dawasir population (LD_{50} = 1.92 ppm). The toxicity results showed that methidathion exhibited similar LD_{50} values in the RPW populations sampled from Al-Ahsa (3.54 ppm), Al-Qatif (3.25 ppm) and Wadi Ad-Dawasir (3.15 ppm). However, the dose mortality response analysis revealed significant differences in the mortality of the RPW population sampled from Wadi Ad-Dawasar after 2 d (F = 74.26; P < 0.0001) and 4 d (F = 39.98; P < 0.0001) of feeding on an artificial diet supplemented with different doses of methidathion. Significant differences in mortality were also observed in the Al-Qatif population after 2 d (F = 50.16; P < 0.0001) and 4 d (F = 63.32; P < 0.0001) and in the Al-Ahsa population after 2 d (F = 44.65; P < 0.0001), 4 d (F = 90.43; P < 0.0001), and 6 d (F = 4.57; P < 0.0118) of feeding on the methidathion-supplemented diet. All of these results are shown in Fig. 1.

The dose-mortality bioassays performed on the Al-Ahsa population of RPWs revealed an increased tolerance to cypermethrin (Fig. 2a), with significant differences obtained 2 d (F = 58.70; P < 0.0001) and 4 d (F = 85.19; P < 0.0001) post-exposure. Similarly, the RPW population from Al-Qatif showed significant differences after 2 d (F = 66.56; P< 0.0001) and 4 d (F = 52.86; P < 0.0001), whereas the Wadi Ad-Dawasir population showed significant differences after 2 d (F = 87.82; P < 0.0001) and 4 d (F=48.23; P < 0.0001) of feeding on the artificial diets containing different doses of cypermethrin (Fig. 2b and c).

Among the tested insecticides, ethion required the maximum dose to attain the LD_{50} value. However, the dose-mortality response bioassays revealed significant differences in the mortality of the RPW population sampled from Wadi Ad-Dawasar after 2 d (F = 81.56; P < 0.0001) and 4 d (F=102.12; P < 0.0001). Significant differences were also observed in the Al-Qatif population after 2 d (F = 58.15; P<0.0001) and 4 d (F = 35.89; P < 0.0001) and in the Al-Ahsa population after 2 d (F = 49.66; P < 0.0001) and 4 d

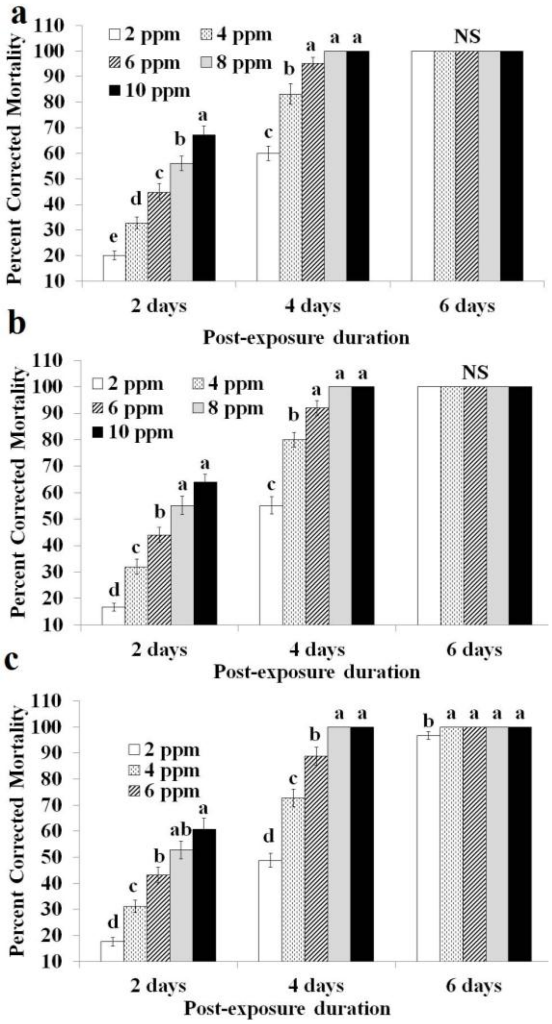

Fig. 1: Influence of methidathion on the average corrected percent mortality of eighth-instar *Rhynchophorus ferrugineus* larvae collected from Wadi Ad-Dawasar (a), Al-Qatif (b), and Al-Ahsa (c). The means ± SE values on the same day that are followed by different letter(s) denote significantly different results, as determined by Fisher's LSD test with α = 0.05

(F = 40.50; P < 0.0001) of being fed the artificial diets containing different doses of ethion (Fig. 3).

Impact of Insecticides on the Nutritional Indices of Different Populations of *R. ferrugineus* Larvae

RPW larvae fed the artificial diet supplemented with insecticides showed significantly affected nutritional indices. The efficacies of the conversion of ingested food (ECI) of the RPW populations sampled from different locations and fed the artificial diet supplemented with methidathion (F = 1619.83; P < 0.0001), cypermethrin (F=362.09; P < 0.0001), and ethion (F = 321.50; P<0.0001) showed significant reductions compared with the control

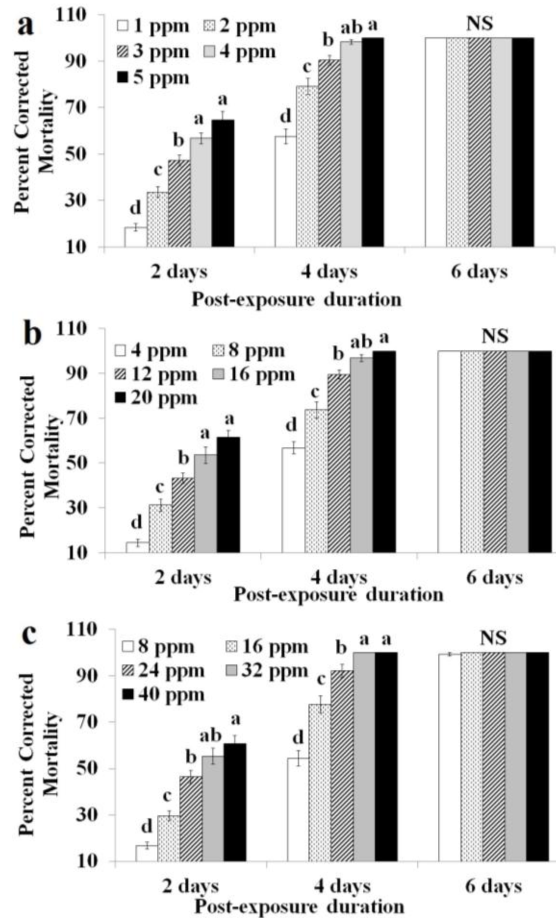

Fig. 2: Influence of cypermethrin on average corrected percent mortality of eighth-instar *Rhynchophorus ferrugineus* larvae collected from Wadi Ad-Dawasar (a), Al-Qatif (b), and Al-Ahsa (c). The means ± SE values on the same day that are followed by different letter(s) denote significantly different results, as determined by Fisher's LSD test with α = 0.05

larvae (Fig. 4). Overall, the maximum reduction (42-46%) in the ECI compared with the controls was observed in the larvae fed methidathion. Relatively smaller reductions (18-22%) were observed in the populations from Al-Ahsa fed diets supplemented with either ethion or cypermethrin.

Moreover, the incorporation of ethion (F = 347.80; P<0.0001), methidathion (F = 2333.62; P < 0.0001), and cypermethrin (F = 428.03; P < 0.0001) into the diets significantly declined the efficacy of conversion of digested food (ECD) in all of the tested populations (Fig. 5). Overall, the maximum reduction (52-57%) was observed in the larvae fed on methidathion. Relatively smaller reductions were observed in the resistant population from Al-Ahsa fed diets supplemented with either ethion (29.93%) or cypermethrin (23.64%). However, the RPW population sampled from Wadi Ad-Dawasar, which was considered the susceptible strain, showed tremendous sensitivity to both

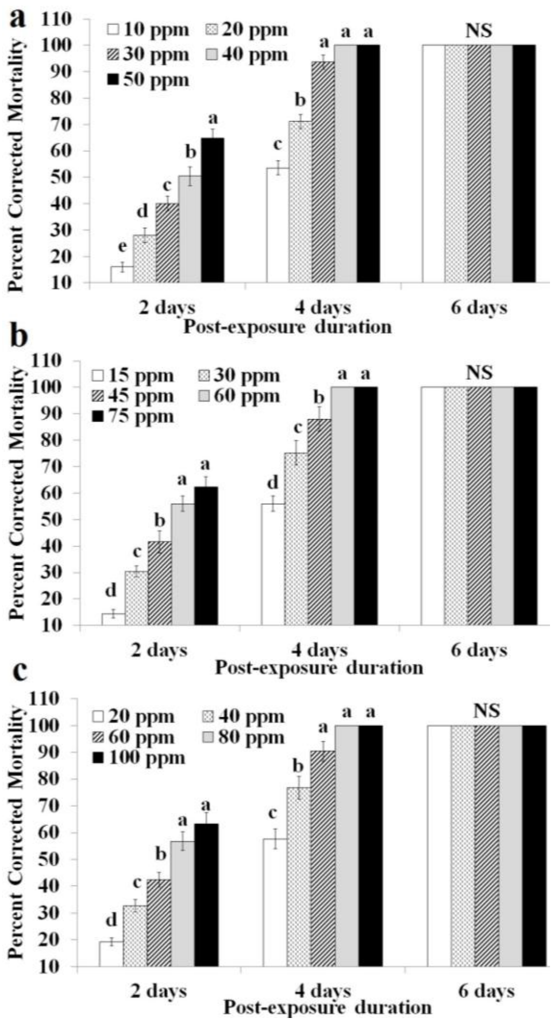

Fig. 3: Influence of ethion on the average corrected percent mortality of eighth-instar *R.hynchophorus ferrugineus* larvae collected from Wadi Ad-Dawasar (a), Al-Qatif (b), and Al-Ahsa (c). The means ± SE values on the same day that are followed by different letter(s) denote significantly different results, as determined by Fisher's LSD test with α= 0.05

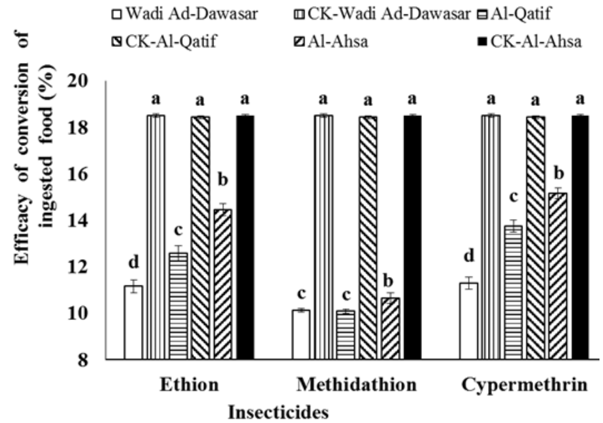

Fig. 4: Efficacy of conversion of ingested food (%) obtained for different populations of *Rhynchophorus ferrugineus* larvae fed artificial diets supplemented with different insecticides. The values present the means from five replicates (n = 25). The means ± SE values for the same insecticide that are followed by different letter(s) denote significantly different results, as determined using Fisher's LSD test with α = 0.05

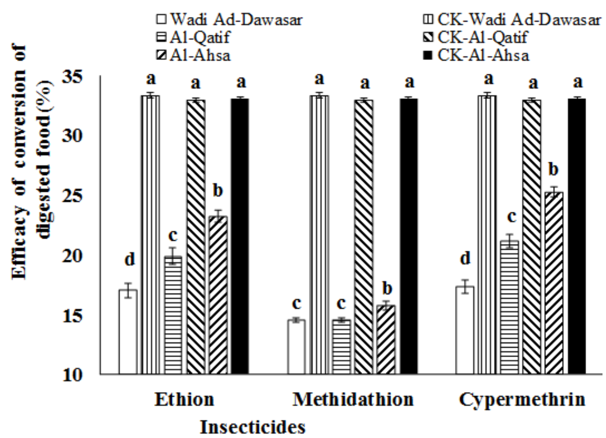

Fig. 5: Efficacy of conversion of digested food (%) obtained for different populations of *Rhynchophorus ferrugineus* larvae fed artificial diets supplemented with different insecticides. The values present the means from five replicates (n = 25). The means ± SE values for the same insecticide that are followed by different letter(s) denote significantly different results, as determined using Fisher's LSD test with α = 0.05

ethion and cypermethrin, showing reductions in the ECD index of 48.99% (ethion) and 48.07% (cypermethrin) compared with the control.

Detoxifying Enzyme Activities

Significant increases in the glutathione S-transferase activity were found in the different RPW populations fed the artificial diets supplemented with either ethion ($F = 123.46$; df = 2, 8; $P < 0.0001$) or cypermethrin ($F = 354.79$; $P<0.0001$). The resistant populations showed tremendous increases in the activity of GST. The RPW population sampled from Al-Ahsa was shown to be resistant to both ethion and cypermethrin, showing 43.46% and 71.43% increases in GST activity relative to the controls, respectively (Fig. 6). Comparatively less GST activity was observed in the RPWs sampled from Al-Qatif and fed either ethion (28.81%) or cypermethrin (48.68%). However, the population from Wadi Ad-Dawasar was found to be the most susceptible population of *R. ferrugineus*, showing only a slight enhancement in GST activity (Fig. 6). The artificial diet supplemented with methidathion did not induce GST activity, resulting in insignificant differences between all of the tested *R. ferrugineus* populations.

Table 1: Susceptibility of three different populations of *Rhynchophorus ferrugineus* to selected insecticides

Insecticide	Population	LD_{50} (95% CL)[a] (ppm)	χ2	Slope ± SE	Resistance ratio[b]
Cyprermethrin	Al-Ahsa	14.13 (12.30-16.23)	5.32	2.32 ± 0.25	8.72
	Al-Qatif	7.30 (6.38-8.35)	5.76	2.33 ± 0.25	4.51
	Wadi Ad-Dawasir	1.62 (1.39-1.88)	7.28	2.27 ± 0.25	1
Methidathion	Al-Ahsa	3.54 (3.10-4.04)	6.22	2.45 ± 0.25	1.12
	Al-Qatif	3.25 (2.81-3.76)	6.60	2.37 ± 0.25	1.03
	Wadi Ad-Dawasir	3.15 (2.74-3.61)	7.42	2.55 ± 0.26	1
Ethion	Al-Ahsa	34.34 (30.10-39.18)	4.55	2.51 ± 0.26	3.77
	Al-Qatif	25.99 (22.67-29.79)	5.06	2.39 ± 0.25	2.85
	Wadi Ad-Dawasir	9.12 (8.15-10.21)	6.85	2.86 ± 0.27	1

[a]95% Confidence limit

[b]Resistance ratio = LD_{50} of the resistant population/LD_{50} of the susceptible (Wadi Ad-Dawasir) population

Discussion

We obtained *R. ferrugineus* populations from major areas of date palm production in the eastern province of the Kingdom of Saudi Arabia and tested these populations for their resistance to three insecticides. Although insecticides have been used extensively against *R. ferrugineus* in Saudi Arabian areas of date palm production for decades, the present report details the first investigation conducted in this country that monitors insecticide resistance in *R. ferrugineus*. Three laboratory populations of *R. ferrugineus* were established from field collections obtained from Wadi Ad-Dawasar, Al-Qatif and Al-Ahsa. These strains showed significant differences in their susceptibility to ethion and cypermethrin through dose-mortality bioassays.

Our laboratory bioassays revealed that the RPW population collected from Wadi Ad-Dawasar is the most susceptible to all of the tested insecticides (Table 1). Therefore, the Wadi Ad-Dawasar population was chosen as the reference strain. Methidathion was found to the most effective insecticide, and none of the populations tested showed resistance. The LD_{50} values of methidathion in all of the populations were markedly lower than the rest of the insecticides. Furthermore, nutritional analyses following the administration of methidathion to *R. ferrugineus* via a supplemented artificial diet revealed significant reductions in the ECI and ECD.

The tremendous decreases in the ECI and ECD values observed in all of the tested *R. ferrugineus* populations compared with their respective controls suggest that less food was being utilized for larval growth and that most of the digested food was metabolized for energy production. Martinez *et al.* (2012) also generated similar results from the evaluation of the insecticidal potential of a lectin-like protein (labramin) in *Ephestia kuehniella*. Similar growth patterns in the presence of toxic compounds have been obtained in insects (Farrar *et al.*, 1989; Weeler and Isman, 2001). In addition, the reductions in the ECI and ECD values may be due to the consumption of methidathion by the larvae because methidathion acts as a stomach poison that interrupts the conversion of digested food into biomass.

The biochemical analyses performed in all of the tested RPW populations revealed similar GST activities.

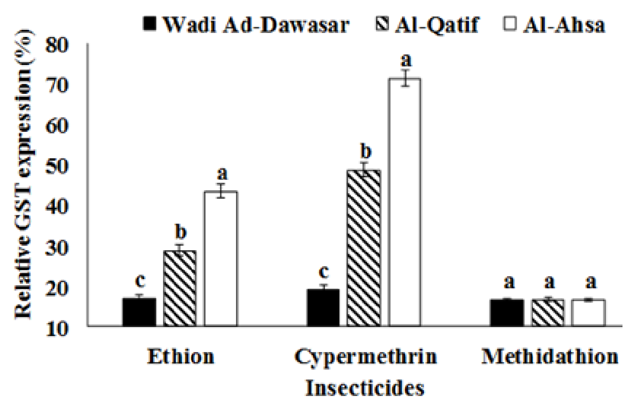

Fig. 6: GST activities (%) of different populations of *Rhynchophorus ferrugineus* larvae fed artificial diets supplemented with different insecticides relative to the control. The values present the means from five replicates. The means ± SE values for the same insecticide that are followed by different letter(s) denote significantly different results, as determined using Fisher's LSD test with α=0.05

Interestingly, the toxicity bioassays, nutritional analyses and biochemical analyses (GST) performed in this study revealed that none of the three tested RPW populations showed any sign of resistance against methidathion.

The inclusion of cypermethrin in the artificial diet fed to *R. ferrugineus* larvae caused significant mortality in a dose-dependent manner. However, differences were observed in the LD_{50} values calculated for all of the tested populations of *R. ferrugineus* larvae after three days. The minimum LD_{50} value (1.62 ppm) was obtained for the population from Wadi Ad-Dawasir. However, resistance to cypermethrin was also observed in two populations of *R. ferrugineus*, including Al-Ahsa and Al-Qatif. The current investigation provides the first report of cypermethrin resistance in these strains. The *R. ferrugineus* population collected from the main areas of date palm growth, where the application of insecticides has peaked, showed the highest resistance to cypermethrin. This level of cypermethrin resistance is not surprising because this insecticide has been intensively used in this region. The variation in the toxicity of cypermethrin among the different populations was further confirmed through dietary utilization bioassays. The incorporation of 1.62 ppm of cypermethrin into the artificial diet markedly reduced the dietary utilization index (ECI) of the Wadi Ad-Dawasar, Al-Ahsa and Al-Qatif populations of RPW by 38.86%, 25.42% and 18.00%, respectively. The reductions in the ECI index obtained in this study are in line with the findings obtained by Koul *et al.* (1996), who reported that some allelochemicals exert strong growth regulatory effects against *Spodoptera litura*. Similarly, the resistant populations from Al-Ahsa and Al-Qatif also showed similar reductions in the ECD, as shown in Fig. 5. The reduced ECI and ECD values obtained for both the resistant and susceptible populations of RPW reveal that the host has to spend most of its energy on defence, leaving only a slight amount of nutrition for growth and development.

An intensive use of cypermethrin around the world has resulted in the development of resistance in arthropod pests. A previous estimate showed that approximately 48 different pest species have developed resistance to cypermethrin (http://www.pesticideresistance.org/search.php; Accessed 22 March 2015). This manuscript presents the first report of cypermethrin-resistant *R. ferrugineus*. These results were further confirmed by exploring the biochemical mechanism underlying resistance development. In this regard, the role of GST in the resistance of RPW to cypermethrin was investigated, and the results showed that GST activity in both of the cypermethrin-resistant strains (Al-Ahsa and Al-Qatif) of *R. ferrugineus* was significantly higher that of the GST activity in the susceptible strain (Wadi Ad-Dawasar). This result suggests that enhanced GST activity is likely to be a major factor that confers cypermethrin resistance to *R. ferrugineus*. Enhanced GST activity has also been shown to be associated with insecticide resistance in *Leptinotarsa decemlineata* and *T. urticae* (Argentine *et al.*, 1992; Stumpf and Nauen, 2002). Cypermethrin, an insecticide to which several species have shown moderate resistance, should no longer be used in the Kingdom of Saudi Arabia and should be replacedwithotherpotent insecticides, including methidathion.

Compared with cypermethrin, ethion showed low resistance in the populations from Al-Ahsa and Al-Qatif. This insecticide is registered and marketed against *R. ferrugineus* in the Kingdom of Saudi Arabia. The toxicity bioassay results revealed signs that ethion resistance has developed in *R. ferrugineus* populations collected from Al-Ahsa and Al-Qatif. The calculated RR_{50} values for ethion showed increases in the Al-Ahsa (3.77-fold) and Al-Qatif (2.85-fold) populations of *R. ferrugineus* after three days. The data generated from the dietary utilization experiments further strengthen our findings by showing smaller reductions in the ECI and ECD indexes in the resistant populations compared with the susceptible population from Wadi Ad-Dawasar (Fig. 4 and 5).

A biochemical analysis of the detoxifying enzyme GST showed elevated levels of GST activity in the larvae from resistant populations of *R. ferrugineus* that were fed an ethion-supplemented diet. The Wadi Ad-Dawasar population (susceptible strain) showed the lowest GST activity. The elevated levels of GST activities suggest the involvement of GST in ethion resistance. The level of ethion resistance detected in the populations from Al-Ahsa and Al-Qatif suggest that the establishment of ethion resistance in the RPW populations is in its initial stages. The role of GST in resistance to synthetic insecticides has been previously evidenced in many pests, including *Aedes aegypti* (Lumjuan *et al.*, 2005), *Anopheles gambiae* (Ortelli *et al.,* 2003), *Bemisia tabaci* (Wang and Wu, 2007), *L. decemlineata* (Argentine *et al.*, 1992), *Liriomyza sativae* (Wei *et al.*, 2014), *Musca domestica* and *Tetranychus urticae* (Kim *et al.*, 2004).

Conclusion

R. ferrugineus populations from Al-Ahsa and Al-Qatif in Saudi Arabia exhibit reduced responses to cypermethrin and ethion that have resulted in the development of resistance to these insecticides. The elevated levels of the detoxifying enzyme GST in the resistant strains of *R. ferrugineus* showed that biochemical resistance may play an important role in the manifestation of insecticide resistance in *R. ferrugineus*. In the future, further studies are needed to fully understand the molecular mechanisms underlying resistance development and to develop strategies for mitigating the resistance problem in order to prevent or delay further increases in insecticide resistance in *R. ferrugineus*.

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References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*., 18: 265–267

Abdul-salam, K.S., M.S. Shawir, M.M. Abo-El-Saad, M.A. Rezk and A.M. Ajlan, 2001. Regent (fipronil) as a candidate insecticide to control red palm weevil, *Rhynchophorus ferrugineus* (Olivier). *Ann. Agric. Sci*., 46: 841–849

Abo-El-Saad, M.M., A.M. Ajlan, M.S. Shawir, K.S. Abdul-salam and M.A. Rezk, 2001. Comparative toxicity of four pyrethroid insecticides against red palm weevil, *Rhynchophorus ferrugineus* (Olivier) under laboratory conditions. *J. Pest Cont. Environ. Sci*., 9: 63–76

Ajlan, A.M., M.S. Shawir, M. Abo-El-Saad, M.A. Rezk and K.S. Abdul-salam, 2000. Laboratory evaluation of certain organophosphorus insecticides against the red palm weevil, *Rhynchophorus ferrugineus* (Olivier). *Sci. J. King Faisal Univ.,* 1: 15–26

Al-Jabr, A.M., M. Rizwan-ul-Haq, A. Hussain, A.I. Al-Mubarak and H.Y. Al-Ayed, 2013. Establishing midgut stem cell culture from *Rhynchophorus ferrugineus* (Olivier) and toxicity assessment against 10 different insecticides. *In Vitro Cell. Dev. Biol. Anim*., 50: 296–303

Al-Rajhy, D.H., H.I. Hussein and A.M.A. Al-Shawaf, 2005. Insecticidal activity of carbaryl and its mixture with piperonylbutoxide against the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) and their effects on acetylcholinesterase activity. *Pak. J. Biol. Sci*., 8: 679–682

Argentine, J.A., J.M. Clark and H. Lin, 1992. Genetics and biochemical mechanisms of abamectin resistance in two isogenic strains of Colorado potato beetle. *Pestic. Biochem. Physiol*., 44: 191–207

Cabello, T.P., J. de la Peña, P. Barranco and J. Belda, 1997. Laboratory evaluation of imidacloprid and oxamyl against *Rhynchophorus ferrugineus*. *Tests of Agrochemicals and Cultivars*., 18: 6–7

El-Mergawy, R.A.A.M., A.M.A.L. Ajlan, N.A. Abdallah, M.I. Nasr and J.F. Silvain, 2011. Determination of different geographical populations of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) using RAPD-PCR. *Int. J. Agric. Biol*., 13: 227–232

El-Sabea, A.M.R., J.R. Faleiro and M.M. Abo-El-Saad, 2009. The threat of red palm weevil *Rhynchophorus ferrugineus* to date plantations of the Gulf region in the Middle-East: An economic perspective. *Outlooks Pest Manage*., 20: 131–134

Farrar, R.R., J.D. Barbour and G.G. Kennedy, 1989. Quantifying food consumption and growth in insects. *Ann*. *Entomol. Soc. Amer*., 82: 593–598

Gadelhak, G.G. and M.R. Enan, 2005. Genetic diversity among populations of red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), determined by Random Amplified Polymorphic DNA-Polymerase chain reaction (RAPD-PCR). *Int. J. Agric. Biol*., 7: 395–399

Habig, W.H. and W.B. Jakoby, 1981. Assays for differentiation of glutathione S-transferases. *Method. Enzymol*., 77: 398–405

Hussain, A., M. Rizwan-ul-Haq and A.M. Al-Jabr, 2013a. Red palm weevil: Understanding the fungal disease mechanism and host defense. *In*: *Microbial Pathogens and Strategies for Combating them: Science, Technology and Education*, pp: 1278-1286. A. Mendez-Vilas (ed.). Formatex Research Center, Spain

Hussain, A., M. Rizwan-ul-Haq, A.M. Al-Jabr and H.Y. Al-Ayied, 2013b. Managing invasive populations of red palm weevil: A worldwide perspective. *J. Food Agric. Environ*., 11: 456–463

Hussain, A., M. Rizwan-ul-Haq, H. Al-Ayedh, S. Ahmed and A.M. Al-Jabr, 2015. Effect of *Beauveria bassiana* infection on the feeding performance and antioxidant defence of red palm weevil, *Rhynchophorus ferrugineus* Olivier (Curculionidae: Coleoptera). *BioControl*., Available online

Hussain, A., M.Y. Tian, Y.R. He and S. Ahmed, 2009. Entomopathogenic fungi disturbed the larval growth and feeding performance of *Ocinara varians* Walker (Lepidoptera: Bombycidae) Larvae. *Insect Sci*., 16: 511–517

Kaakeh, W., 2006. Toxicity of imidacloprid to developmental stages of *Rhynchophorus ferrugineus* (Curculionidae: Coleoptera): Laboratory and field tests. *Crop Prot*., 25: 432–439

Kim, Y.J., S.H. Lee, S.W. Lee and Y.J. Ahn, 2004. Fenpyroximate resistance in *Tetranychus urticae* (Acari: Tetranychidae): cross-resistance and biochemical resistance mechanisms. *Pest Manage. Sci.*, 60: 1001–1006

Koul, O., J.S. Shankar and R.S. Kapil, 1996. The effect of neem allelochemicals on nutritional physiology of larval *Spodoptera litura*. *Entomol. Exp. Appl*., 79: 43–50

Llácer, E. and J.A. Jacas, 2010. Short communication. Efficacy of phosphine as a fumigant against *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in palms. *Span. J. Agric. Res*., 8: 775–779

Lumjuan, N., L. McCarroll, L.A. Prapanthadara, J. Hemingway and H. Ranson, 2005. Elevated activity of an Epsilon class glutathione transferase confers DDT resistance in the dengue vector, *Aedes aegypti*. *Insect Biochem. Mol. Biol*., 35: 861–871

Martinez, D.S., Md. Freire, P. Mazzafera, R.T. Araujo-Júnior, R.D. Bueno and M.L. Macedo, 2012. Insecticidal effect of labramin, a lectin–like protein isolated from seeds of the beach apricot tree, *Labramia bojeri*, on the Mediterranean flour moth, *Ephestia kuehniella*. *J. Insect Sci*., 12: 62

Ortelli, F., L.C. Rossiter, J. Vontas, H. Ranson, J. Hemingway, 2003. Heterologous expression of four glutathione transferase genes genetically linked to a major insecticide-resistance locus from the malaria vector *Anopheles gambiae*. *Biochem J*., 373: 957–963

SAS Institute, 2000. SAS user's guide: statistics, SAS Institute, Cary, North Carolina, USA

Shar, M.U., M.A. Rustamani, S.M. Nizamani and L.A. Bhutto, 2012. Red palm weevil (*Rhynchophorus ferrugineus* Olivier) infestation and its chemical control in Sindh province of Pakistan. *Afr. J. Agric. Res*., 7: 1666–1673

Stumpf, N. and R. Nauen, 2002. Biochemical markers linked to abamectin resistance in *Tetranychus urticae* (Acari: Tetranychidae*). Pestic. Biochem. Physiol*., 72: 111–121

Wang, L.H. and Y.D. Wu, 2007. Cross-resistance and biochemical mechanisms of abamectin resistance in the B-type *Bemisia tabaci*. *J. Appl. Entomol*., 131: 98–103

Weeler, D.A. and M.B. Isman, 2001. Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*. *Entomol. Exp. Appl*., 98: 9–16

Wei, Q.B., Z.R. Lei, R. Nauen, D.C. Cai and Y.L. Gao, 2014. Abamectin resistance in strains of vegetable leafminer, *Liriomyza sativae* (Diptera: Agromyzidae) is linked to elevated glutathione S-transferase activity. *Insect Sci*., 22: 243–250

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